The Effects of Pregnancy on Biliary Lipids in Rhesus Monkeys (36091)

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A marked alteration in plasma lipid levels occurs during gestation in the rhesus monkey (1). Cholesterol, phospholipids, and total lipids decrease after week 5, with a maximum depression occurring between weeks 9 and 13. Free fatty acids and triglycerides fluctuate randomly until the last month of pregnancy, when an elevation is noted. Normal values of all moieties are regained within 1 month postpartum. No explanation is presently available for these lipid changes. Since the liver and gallbladder play a major role in the synthesis, storage, and metabolism of lipids, it was of interest to determine the extent to which the marked blood lipid alterations in the monkey could be explained on the basis of an altered biliary excretion of lipids.

In the human, liver function is known to be altered during pregnancy and suggestive (2), though by no means conclusive, evidence indicates a biliary stasis with resulting gallstone formation. In some women, pregnancy or the administration of ethinyl estradiol has been known to induce a cholestatic jaundice (3). Virtually no information is available from other species on this suggested relationship between pregnancy and biliary function.

Methods. A total of 14 rhesus monkeys was used in this study, all of which were regularly cycling females maintained in the colony as previously described (4). Peripheral blood and gallbladder bile were obtained at laparotomy. Six animals subsequently were mated and all became pregnant. From four of these monkeys, samples of peripheral blood and gallbladder bile were obtained at laparotomy during weeks 5, 9, 13, 17, and 21 of gestation, and 1 month postpartum. The other two animals aborted early in pregnancy. In addition, in the four monkeys that had a normal gestation, a peripheral blood sample was obtained within 12 hr after delivery.

All monkeys were anesthetized with sodium pentobarbital (Diabutal, Diamond Laboratories, Inc.) or sodium thiamylal (Surital, Parke, Davis & Co.). With the exception of the animals which were mated, the gallbladder was evacuated, and an average of 3 ml of bile was removed. From the mated animals, 1 ml of bile was taken routinely at each laparotomy. After the blood was centrifuged and the plasma was removed, both plasma and bile were stored at −17° until analyzed. This low storage temperature was considered a necessary precaution to minimize autolytic changes that have been reported to occur in bile (5).

Total lipids, including bile salts, were determined by the method of Bragdon (6), and phospholipids were determined as lipid phosphorus using a modification of the method of Chen *et al.* (7), replacing 70% $HClO_4$ with 30% H_2O_2 (0.0005% phosphorus contamination) in the digestion phase. Plasma total cholesterol was determined by the procedure of Bowman and Wolf (8). Using this method, the bile salts in plasma form a chromogen having a peak absorbance at 380 mμ, and their low concentration in peripheral plasma does not interfere with the cholesterol peak at 550 mμ. In bile, however, the bile salt concentration is so high that the 550 mμ peak of cholesterol is usually masked as part of the shoulder of the 380 mμ peak. Hence, bile salts were first separated from the ethanolic extraction mixture by thin-layer chromatography, using glass plates coated with heat-activated silica gel F254 in a layer thickness of 0.25 mm (Brinkman Instruments). The solvent consisted of a 150:50:4 (v:v:v) mixture of chloroform, methanol,

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TABLE I. Lipid Constituents of Normal Rhesus Monkey Plasma and Gallbladder Bile.[a]

Constituent	Conc (mg/100 ml)
Plasma cholesterol	144.8 ± 8.5
phospholipids	229.5 ± 9.1
total lipids	495.3 ± 29.6
Biliary cholesterol	337.9 ± 49.6
phospholipids	3079 ± 351
total lipids	6508 ± 593
bile salts	3091 ± 323[b]

[a] Mean values ± SEM from 14 monkeys.

[b] Calculated estimate of mean ± SEM based upon evidence from Thureborn (9) that cholesterol, phospholipids, and bile salts comprise essentially all of the biliary lipid content.

and water. The cholesterol band moved with the solvent front, whereas the bile salts in this system had a $R_F = 0.35$. Elution of the cholesterol was completed using a 2:1 (v:v) mixture of chloroform and methanol; and, after evaporation to dryness, the sample was analyzed according to the Bowman–Wolf (8) procedure with no further modifications.

Results and Discussion. Plasma and biliary lipid data from the 14 normal female monkeys are presented in Table I. In other species, it is known (9) that the gallbladder concentrates lipids, and the control data presented indicate that this also occurs in the rhesus monkey. Biliary cholesterol levels are more than twice as high as plasma levels; phospholipids and total lipids are concentrated by a factor of more than 13. By comparison with human data (2), one can conclude that in the two species, these moieties are concentrated very similarly except for cholesterol. Human biliary cholesterol is approximately 1.5 times that found in the monkey. Since the solubility of cholesterol in bile is dependent on the interaction of phospholipids and bile salts (10), the difference in cholesterol levels in the two species may contribute to the low incidence of gallstones observed in rhesus monkeys.

Table II summarizes the plasma and biliary data obtained from the four animals which completed pregnancy. Plasma cholesterol levels are significantly depressed from that seen in nonpregnant monkeys, during weeks 9 ($p<.01$), 13 ($p<.05$), and 17 ($p<.01$) of gestation; whereas biliary cholesterol levels do not change significantly at any of these times. Plasma phospholipid levels are significantly lowered from control values at weeks 9 ($p<.02$), and 13 ($p<.02$); but again, biliary phospholipid levels are not significantly altered. Plasma total lipids are significantly less than nonpregnant concentrations at weeks 9 ($p<.01$), 13 ($p<.01$), and 17 ($p<.01$). These lipid alterations are similar to those reported previously (1). However, none of the measured biliary lipid moieties appear to be altered significantly from nonpregnant controls.

These data suggest that, in the rhesus monkey, an increased biliary concentration of lipids during pregnancy does not occur, and

TABLE II. Plasma and Gallbladder Bile Lipid Alterations During Pregnancy in the Rhesus Monkey.[a]

	Plasma			Bile		
	Cholesterol	Phospholipids	Total lipids	Cholesterol	Phospholipids	Total lipids
Control	141.6 ± 10.1	227.1 ± 13.6	556.6 ± 37.5	506.8 ± 119.7	3236 ± 692	7180 ± 1081
Pregnancy						
week 5	130.7 ± 12.6	215.1 ± 18.3	441.5 ± 47.2	482.3 ± 112.1	3088 ± 363	7807 ± 1612
9	76.2 ± 13.2	133.1 ± 26.4	324.6 ± 38.8	400.1 ± 107.9	2767 ± 834	5494 ± 504
13	82.4 ± 16.9	120.8 ± 26.5	257.0 ± 46.1	491.7 ± 135.3	3128 ± 856	6574 ± 1053
17	72.6 ± 10.1	147.5 ± 36.8	258.7 ± 45.1	496.7 ± 163.4	2915 ± 705	5343 ± 828
21	105.1 ± 29.9	199.9 ± 45.9	461.6 ± 104.7	461.2 ± 57.4	2718 ± 503	5710 ± 468
Delivery	97.9 ± 23.7	193.3 ± 47.9	365.8 ± 105.3	—	—	—
Postpartum 1 month	157.3 ± 10.4	257.5 ± 17.0	479.9 ± 20.4	446.1 ± 100.4	2615 ± 596	6305 ± 600

[a] Mean values (mg/100 ml) ± SEM from 4 monkeys.

as such does not explain the depressed plasma lipid levels occurring at this same time. The possibility of an increased bile volume due to elevated production and flow with no change in biliary lipid concentration cannot be ignored, however.

It is known that during human pregnancy, liver function is altered, as shown by the BSP test (11). The liver takes up BSP more rapidly and doubles its return into plasma from parenchymal cells. However, there is a decrease in the rate of excretion of BSP into the bile. The relationships between the rates of hepatic conversion of cholesterol into bile salts and BSP excretion into the biliary system are not known in either the human or the monkey.

Summary. In an effort to determine whether alterations in biliary excretion of lipids could aid in explaining the marked hypolipemia seen during pregnancy in the rhesus monkey, the concentrations of cholesterol, phospholipids, and total lipids were measured at selected stages of gestation. No significant changes in biliary lipid levels were observed, indicating that increased biliary concentration of lipids probably does not occur during pregnancy.

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